Evaluation of Restriction Endonuclease Analysis as an Epidemiologic Typing System for Branhamella catarrhalis

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Restriction endonuclease analysis (REA) was evaluated as an epidemiologic typing tool to distinguish Branhamella catarrhalis strains. Fourteen β-lactamase-producing strains were collected over a 16-month period at a hospital where a nosocomial outbreak of this organism was previously documented by REA. REA produced 12 distinct patterns which correlated with epidemiologic data. Chromosomal REA appears to be a useful technique for distinguishing B. catarrhalis strains.

Branhamella catarrhalis has become an increasingly recognized respiratory pathogen, particularly in patients with compromised respiratory tract function (10). Most cases of B. catarrhalis infection are sporadic, but nosocomial spread of the organism has been suggested (1, 2, 7). Documentation of nosocomial transmission of B. catarrhalis has been difficult due to the lack of a typing system to confirm strain identity. Recently, we documented a nosocomial outbreak of B. catarrhalis respiratory infection in the intermediate care unit of a Veterans Administration hospital; restriction endonuclease analysis (REA) of chromosomal DNA was used to confirm strain identity (11). In that study, five patients and two staff members were infected or colonized with the outbreak strain. The current study further evaluates the utility of chromosomal restriction endonuclease digestion as a marker of B. catarrhalis strain identity by studying non-epidemic isolates in the hospital where the outbreak occurred.

(This work was previously presented and published in abstract form [J. E. Patterson, T. F. Patterson, B. Masecar, W. J. Hierholzer, Jr., and M. J. Zervos, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, L-11, p. 412].)

MATERIALS AND METHODS

Strain collection and microbiologic and epidemiologic data. Thirteen β-lactamase-producing B. catarrhalis strains isolated from sputum specimens as the sole or predominant pathogen were collected from the West Haven Veterans Medical Center Microbiology Laboratory over a 16-month period. Duplicate isolates from the same patient were excluded. Nine consecutive strains were collected during the time of the outbreak from January to February 1987. The seven identical outbreak strains which have been previously reported (11) were excluded from this study, except for one strain which was used for comparison. Three strains were collected from April to June 1987, and one strain was collected 1 year later in April 1988. Isolates were identified as B. catarrhalis on the basis of characteristic growth and biochemical reactions (4). β-Lactamase was detected by using nitrocefin disks (BBL Microbiology Systems, Cockeysville, Md.). A retrospective chart review was done before

REA results were known to determine the epidemiologic relatedness of strains by patient location, length of stay, and date of isolation.

DNA isolation and restriction endonuclease analysis. B. catarrhalis strains were stored in skim milk at −70°C. For cell lysis, cells were grown at 37°C in a carbon dioxide incubator on blood agar plates. Cells from the plate were then inoculated into 250 ml of brain heart infusion broth for overnight incubation at 37°C with vigorous shaking. Two methods were used for cell lysis. Method 1 was used to examine strains for extrachromosomal DNA. Cells were lysed by using a previously described lysozyme-0.1% Triton X-100 procedure (5). DNA was isolated by cesium chloride-ethidium bromide density gradient centrifugation followed by ethanol precipitation. Method 2 was used to obtain chromosomal DNA for digestion. Cells were lysed by a previously described lysozyme-20% sodium dodecyl sulfate procedure (6). DNA was purified using phenol-chloroform extractions followed by ethanol precipitation (6).

Chromosomes were digested with eight restriction endonucleases: HaeIII, HindIII, PstI, Clal, Aval, EcoRI, BglIII, and MspI (BRL, Gaithersburg, Md.). DNA (1.5 μg) was digested according to the specifications of the manufacturer. DNA was then analyzed by 0.5, 0.6, 0.7 and 0.8% agarose-40 mM Tris acetate–1 mM EDTA gel electrophoresis on a horizontal gel apparatus (8) at 40 V for 8 h.

RESULTS

Distinguishable digestion patterns of chromosomal DNA were produced by HaeIII, HindIII, PstI, and Clal. HaeIII digestion patterns were the most definitive. The 0.5% agarose-40 mM Tris acetate–1 mM EDTA gel gave the best resolution of the distinguishing fragments. Patterns were distinguished by the higher-molecular-weight fragments; smaller fragments were homogeneous. DNA yield from the lysozyme-sodium dodecyl sulfate procedure was 2.5 to 10 μg per 100 ml of cell culture. No plasmid DNA was isolated.

Twelve distinct REA patterns were seen. Nine strains showed heterogeneous REA patterns; each of these was distinct from the pattern of the epidemic isolate (Fig. 1). Two pairs showed identical patterns. The identity of the strains in both pairs was confirmed by digestion with a second restriction endonuclease, Clal.

Medical records of 12 of the 13 patients were available for review. Five of twelve strains were isolated from hospital-
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DISCUSSION

We recently documented nosocomial transmission of B. catarrhalis by using restriction endonuclease analysis (11). However, the lack of a typing system prior to restriction endonuclease analysis of DNA content made the epidemiology of B. catarrhalis difficult to determine. β-Lactamase production is now a common trait in B. catarrhalis strains (11); a previous study has shown that isoelectric focusing of β-lactamase-producing isolates has not been specific enough to distinguish individual strains (9). Bacteriocin typing has been attempted but was not successful in typing strains from a suspected outbreak (1). In general, B. catarrhalis does not contain plasmid DNA, and we isolated none from our strains. Thus, plasmid DNA could not be used to distinguish these strains. Restriction endonuclease analysis of chromosomal DNA has been used to distinguish a number of organisms (13), including Neisseria spp. (12). Among its advantages over analysis of plasmid DNA are that strains which do not contain plasmid DNA can be evaluated and that chromosomal DNA may be a more stable identity marker in strains which lose plasmid DNA over time.

In this study, 10 epidemiologically unrelated strains had distinct digestion patterns. One identical pair of isolates may represent an endemic strain in the long-term care facility of the hospital, since both patients had been hospitalized for more than 2 months on the same ward. Interestingly, the nosocomial outbreak previously reported also occurred in this building used for long-term care but was due to a different transmitted strain (11). Compromised respiratory tract function is common in this population, and many adult B. catarrhalis infections occur in patients with this risk factor (10).

The other identical pair of isolates were cultured from a recently hospitalized patient and from an outpatient. The hospitalized patient had no previous sputum culture from that admission and could have harbored the B. catarrhalis strain on admission. These two patients had no apparent epidemiologic relation except for the possibility that both strains were community acquired. Similar results were demonstrated in a recent study which used restriction endonuclease analysis of whole-cell B. catarrhalis DNA to compare nasopharyngeal and middle ear isolates from children who underwent tympanostomy for otitis media (3). Nasopharyngeal and middle ear isolates were identical in seven of eight cases; isolates from each episode of otitis were distinct except for one pair of identical isolates from apparently epidemiologically unrelated patients. The detection of identical REA patterns in apparently epidemiologically unrelated patients emphasizes the importance of concurrent control strains and epidemiologic information with this or any other typing system.

Restriction endonuclease analysis appears to be a useful marker of strain identity for nonepidemic isolates and certainly appears to be helpful in the outbreak setting. Epidemiologic information must be used in combination with molecular epidemiologic typing results, particularly for long-term or nonepidemic studies.

It is of interest that 7 of the 12 patients in our study had B. catarrhalis isolated from their sputum an average of 38 days after hospital admission. This further suggests the potential importance of B. catarrhalis as a hospital-acquired pathogen. Now that an epidemiologic typing system is available, further surveillance and analysis will aid in the study of the epidemiology of both community and hospital-acquired B. catarrhalis infections.

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